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# Polyelectrolyte Micelles: Self-Diffusion and Electron Microscopy Studies

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We report the results of a study on aqueous solutions of hydrophobically modified polyelectrolytes (charge-neutral diblock copolymers) by different techniques which have been specifically chosen for demonstrating unambiguously the existence of micelles. Self-diffusion measurements by fluorescence recovery after photobleaching (FRAPP) and pulsed NMR spectroscopy measure the micellar sizes which are in agreement with light scattering data on dilute solutions. Cryo-transmission electron microscopy (Cryo-TEM) pictures confirm the existence of the micellar cores in contrast with homopolyelectrolyte solutions where no features are observed.

## Introduction

In contrast to block copolymer micelles in organic solvents, which have been extensively studied and reviewed,[1] the properties of micelles made of block copolymers composed of a long charged block and a short hydrophobic block are not so well-known. However, the importance of such architectures is at least 2-fold. First, the corona of such objects enables one to study tethered polyelectrolytes around a central core and to test various theoretical predictions about charged brushes. Second, such charged diblock copolymers are a new class of surfactants whose general properties (in terms of phase diagrams for instance) are not known. Theory has predicted[2] that asymmetric diblocks (short neutral part/long charged part) should not micellize in water due to the large energy penalty caused by the highly charged corona of the micelle when compared to the energy gained by association in the hydrophobic core.

Recently, another theoretical analysis of this problem,[3] taking into account the condensation of counterions along the chains which mitigates the corona energy penalty, has shown that micellization is possible. Also, a couple of experimental attempts indicate that such an association resulting in forming an urchin-like micelle with a small hydrophobic core surrounded by a long charged corona is probable.[4] We recently[5] reported the existence of micelles in a simple two component system of water plus a highly asymmetric block copolymer: a short poly(*tert*-butylstyrene) hydrophobic block and a long sodium poly(styrenesulfonate) charged block. In particular, quasi-elastic light scattering (QELS) revealed the existence of modes which can be interpreted as diffusive modes of micelles. However, it is known that dynamic modes which are not due to the diffusion of particles can be observed in polyelectrolytes.[6] As a consequence, it is always difficult to reach an unambiguous measure of the dimensions of charged diblocks by QELS studies only. Moreover, even when a diffusive mode is recognized, correlation decay curves are never monoexponential, which makes quantitative interpretation rather delicate.[7]

We show in this paper that this difficulty in assessing the existence and the characteristics of such complicated objects can be removed successfully by the use of alternative methods. A number of techniques have been developed which measure the self-diffusion coefficient of an object. A common feature of these techniques is that individual molecules or objects are made into labeled tracers whose Brownian motion is followed. This eliminates typical artifacts in QELS resulting from its sensitivity to collective properties of mixtures and not individual components. We present in this paper the results from two such techniques: fluorescence recovery after fringe pattern photobleaching (FRAPP) and pulsed field gradient spin−echo NMR spectroscopy. We demonstrate the utility of such methods in this field and show that good quantitative agreement can be reached with previous results obtained by more common means. In addition to the diffusion measurement, we also report the results of cryo-transmission electron microscopy (cryo-TEM). Cryo-TEM has become a standard technique for the visualization of association colloids (e.g., spherical, discoidal, and wormlike micelles) of ordinary surfactants in solution. Although the polyelectrolyte parts of the block

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copolymer surfactants in the micelles are not expected to be close-packed enough to create the contrast needed for TEM, we show that the hydrocarbon cores of the micelles are visible in the same way as conventional micelles have enough contrast to be seen in TEM. In what follows we describe the experimental techniques and discuss our findings in detail.

## Materials and Methods

**Materials.** We made use of diblock copolymers of poly(*tert*-butylstyrene) (PtBS) (hydrophobic block with a degree of polymerization of 25) and sodium poly(styrenesulfonate) NaPSS (polyelectrolyte block with degree of polymerization of 404). This material, designated MT-2, was synthesized by anionic polymerization[8] ($M_w/M_n = 1.03$) and is highly charged (sulfonation of 89%).

**Fluorescence Recovery after Fringe Pattern Photobleaching (FRAPP).** The FRAPP experiment allows measurements of the self-diffusion coefficient $D_s$ of fluorescent probes.[9] When strongly illuminated, these probes lose their fluorescence (photobleaching). In the illuminated volume, the fluorescence intensity is measured afterward by use of a low intensity light beam. The signal increases as new probes diffuse back to the photobleached volume and $D_s$ can be deduced from the recovery time. The sensitivity of the method is improved by use of a fringe pattern and by modulation of the fluorescence signal. The fluorescence recovery curves were fitted to the following expression:

$$V(t) = A\mathrm{e}^{-t/\tau} + B \qquad (1)$$

where $\tau = i^2/4\pi^2 D$ and $i$ is the fringe spacing. For each sample we checked that the time $\tau$ scales with $i^2$ and $D_s$ was deduced from a plot of $\tau^{-1}$ versus $k^2 = (2\pi/i)^2$. Small amounts of a fluorescent probe (3,3′ diethyloxadicarbocyanine iodide or DODCI from Kodak) were added to each solution (typically 5 mg/cm$^3$). Due to its positive charge, we suggest that the fluorescent part of the probe is attached to the negatively charged corona. It was also found necessary to add some quantity of hydrochloric acid (HCl) solution (at least 0.2 cm$^3$ of a $10^{-2}$M HCl solution per cm$^3$ of the polymer solution) in order to make the solution bleach efficiently by a change in pH. Under the above conditions, solutions were irreversibly bleached through a grating of adjustable spacing. The recovery of the fluorescence in the sample was subsequently monitored with time.

**Quasi-Elastic Light Scattering (QELS).** Photon correlation experiments were performed using a custom built correlator and a laser source at a wavelength of 514.5 nm. Autocorrelation functions $g(t)$ of the electric field were found nonexponential. An analysis in terms of cumulants was made in order to estimate the hydrodynamic radius of the micelles. To do so, we either calculate the slope of the mean decay rate $\Gamma$ versus $q^2$ or plot the apparent mutual diffusion coefficient $D_m = \Gamma/q^2$ for high wavevectors $q$ and extrapolate to zero. Here $\Gamma$ is the mean decay rate provided by the convergence of the cumulant analysis. Both measures provide consistent values whose dispersion was used to provide the error bar estimation of our results. We recently showed, using an analogous polymer, that such a procedure was adequate to characterize the micelles, in agreement with an inversion technique such as the Maximum Entropy method[7] (see Figure 2-a of ref 7). Such measurements were performed in polymer solutions of concentration 0.1 wt % with addition of hydrochloric acid (same proportions as for the FRAPP experiment), with addition of hydrochloric acid and DODCI (same proportions as for the FRAPP experiment) and finally, in the very same batches used for the FRAPP experiments. These various measurements at an identical polymer and added electrolyte concentration provide us with an accurate estimation of the error bar on the hydrodynamic radius.

**Pulsed Field Gradient Spin−Echo Nuclear Magnetic Resonance Spectrocopy (NMR Spectroscopy).** Self-diffusion coefficient measurements were performed on a Nicolet NMC 1180 NMR spectrometer operating at 300 MHz for protons. The spin−echo pulsed field gradient technique used here has been described elsewhere.[10] The experiments were done by varying the field gradient pulse duration, $\delta$, with a constant field gradient pulse interval, $\Delta$. The self-diffusion coefficient, $D_s$, analoguous to the one measured by FRAPP, was determined by fitting the decay of the echo amplitude, A, with the equation

$$A = A_o \exp\left[-\gamma_g G^2 D_s \delta^2 (\Delta - \delta/3)\right] \qquad (2)$$

In this expression, $A_o$ is the echo amplitude in the absence of a gradient pulse, $G$ is the field gradient strength, and $\gamma_g$ is the gyromagnetic ratio. The field gradient $G$ was calibrated using neat benzene, whose self-diffusion coefficient is known to be 2.207 $\times 10^{-9}$ m$^2$/s at 25 °C.[11] For the NMR studies, solutions of polymer in $D_2O$ were prepared at different concentrations of NaCl. Care was taken to dry the polymer prior to dissolution. To get a sufficiently strong NMR echo signal $A$, the concentration of the polymer had to be at least 1%. Error bars on $D_s$ were estimated according to the dispersion of results of different runs.

For the three above techniques, the apparent hydrodynamic radii, $R_h$, of micelles were calculated using Stokes law for spheres; namely, $R_h = k_B T/6\pi\eta D$ where $k_B$ is Boltzmann's constant, $T$ the absolute temperature, and $\eta$ the viscosity of water, and $D$ is $D_s$ or $D_m$ according to the measurement (see above).

**Cryo-Transmission Electron Microscopy (Cryo-TEM).** Cryo-TEM samples were prepared in the controlled environment vitrification system (CEVS) described elsewhere.[12] In the CEVS, temperature was controlled to within 0.1 °C and the humidity was controlled to be at nearly 100% saturation to prevent evaporation of the sample. Thin films of sample were prepared by placing a 3 mL drop of solution on a holey polymer carbon coated film mounted on a microscope grid. Two solutions of 0.1 and 1 wt % in pure water were prepared for that purpose. The drop was blotted with filter paper so that thin films (10 to 500 nm) remained spanning the 2−8 $\mu$m diameter holes in the polymer film. The sample was then vitrified by plunging in liquid ethane and transferred and viewed in the TEM with the aid of a liquid nitrogen cold stage which kept the sample in its vitrified state. More details of the technique can be found elsewhere.[13]

## Results and Discussion

**Hydrodynamic Radius of Weakly Interacting Micelles as Evidenced by FRAPP and QELS.** FRAPP recovery curves such as Figure 1 were obtained at various spacing values for different polymer and salt concentrations. From each data set a good fit of the signal showing the fluorescence recovery with time can be obtained by a single exponential. The characteristic time of the exponential is of diffusive nature, i.e., $\tau^{-1}$ is proportional to $k^2$ (see inset in Figure 1), which lead us to attribute this signal to the diffusion of real objects whose large dimensions indicate their associated nature. A comparison with the QELS results is made in Figure 2. The agreement corroborates the qualitative and quantitative trends found by QELS for the hydrodynamic radius $R_h$ of the micelles upon addition of salt. The micelles are shown to contract weakly with increasing salinity, presumably due to the additional screening of the charged corona of the micelles. This behavior has to be contrasted with the significant contraction of homopolyelectrolytes (NaPSS) upon salt addition.[14] The FRAPP and QELS measurements were carried out at polymer concentrations of 0.1 wt %. For

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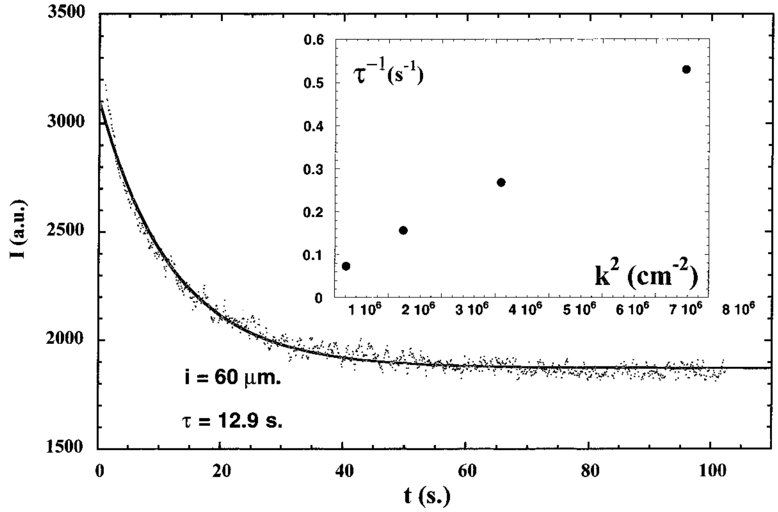

**Figure 1.** Typical fluorescence recovery curve for fringe spacing of 60 $\mu$m fitted by one exponential (squares). The decay time of 12.9 s is interpreted as the diffusion time of the micelles. The inset shows the diffusive nature of the relaxation since $\tau^{-1}$ is linear versus $k^2$.

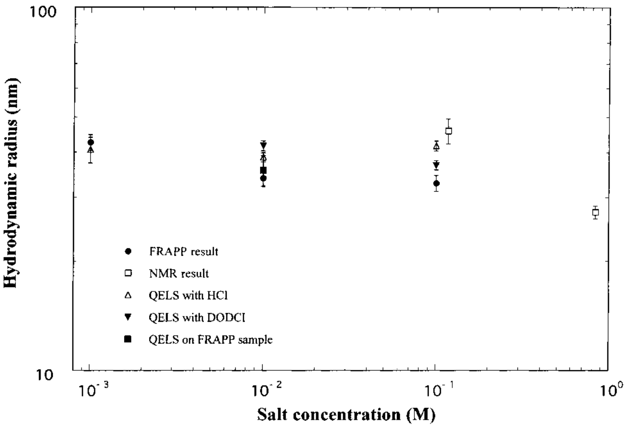

**Figure 2.** Hydrodynamic radius versus salt concentration as determined by the different techniques QELS, FRAPP, and NMR. QELS results with HCl correspond to light scattering when HCl is added to the polymer solution in the same proportions as in the FRAPP sample (empty triangles). QELS results with DODCI correspond to light scattering when HCl and DODCI are added to the polymer solution in the same proportions as in the FRAPP sample (filled inverted triangles). QELS on FRAPP sample refers to light scattering in the same samples as used in FRAPP measurements (filled square).

FRAPP measurements, only salted samples (salinity of $10^{-3}$ M and higher) can be studied due to the necessity of working at a low pH for bleaching the fluorescent probe. In these polymer and salt concentration ranges, micelles are only weakly interacting and the agreement between both techniques shows that the results were both obtained in the asymptotic regime ($c \rightarrow 0$) where $D_s$ and $D_m$ are identical. This can be confirmed by an estimation of the overlap concentration, $c^*$, for such hydrodynamic radii and for an aggregation number of order 30 which was independently estimated by neutron scattering measurement.[5] A value of order $c^* = 1$ wt % is obtained, far above the maximal concentrations used in this study. The typical distance between objects can be estimated at about 80 nm, a distance always larger than the screening length at the lowest salinity (on the order of 10 nm). Consequently, both electrostatic and osmotic interactions must be weak in this range of concentration.

It must be noted that reasonable theories suggest that in these systems a large majority of the counterions stay trapped within the corona, making the effective charge of the micelle weak.[3,15]

Moreover the equality (within error bars) of $D_s$ and $D_m$ indicates a rather weak polydispersity of the micelles since $D_s$ results from a number average whereas $D_m$ results from a $z$ average.[16]

**NMR Measurements of the Hydrodynamic Radius.** Measurements of the hydrodynamic radius by NMR were also obtained (Figure 2), in qualitative agreement with the general trend of weak contraction of the radius with added salt. The necessity of using concentrated samples (1 wt % in polymer) in order to get a sufficient signal-to-noise ratio makes the quantitative comparison between NMR and other measurements more delicate. The high concentration used may be responsible for the slight overestimation of the radius by NMR observed at the lowest salinity of 0.18 M (Figure 2). Since micelles weakly contract upon addition of salt, micelles are still quite close to $c^*$ at this salinity, and interactions (both electrostatic and osmotic) may alter the diffusion coefficient $D_s$ and thus apparently increase $R_h$. Indeed as previously calculated[17] and observed,[18] one generally expects a decrease of $D_s$ with concentration and thus an apparent increase of $R_h$.

Another possibility worth mentioning is the possible change in shape of the micelles from spherical to a more elongated shape. This trend is suggested by one of the CryoTEM pictures taken at a polymer concentration of 1 wt % (see below). We discuss further these two possibilities in the next paragraph.

**Hydrophobic Core of the Micelles Evidenced by CRYOTEM.** The cryo-TEM micrograph shown in Figure 3 provides further support to our interpretation of the diffusion data. A 0.1 wt % polymer solution in pure water was studied. The first point to be made from the micrograph is that in the solution there are certainly aggregates with diameter of several nanometers. Cryo-

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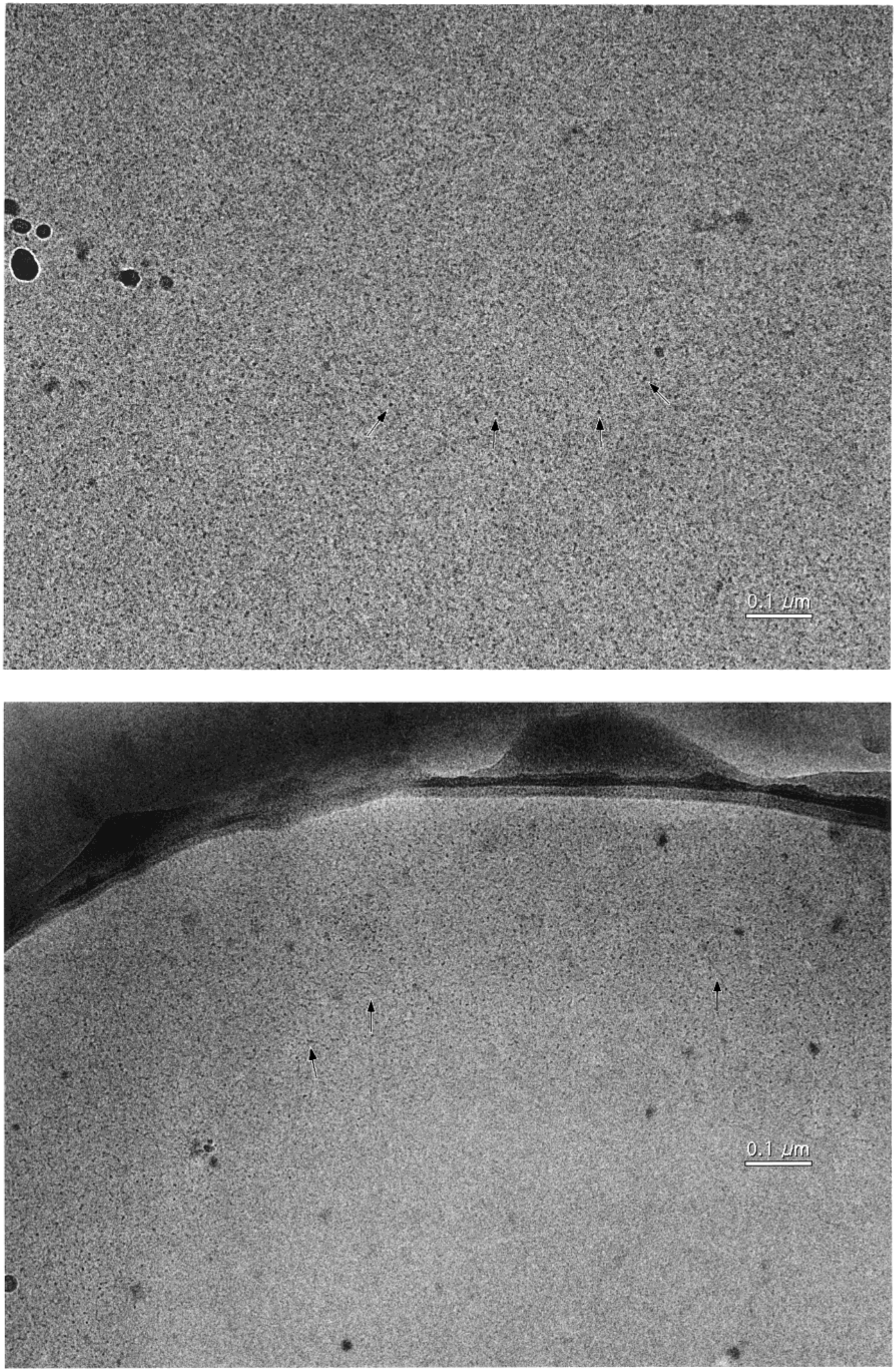

**Figure 3.** Cryo-TEM pictures of polymer solutions (0.1 wt % (a, top) and 1 wt % (b, bottom)). Arrows point to: (a, top) dense hydrophobic cores of the micelles; (b, bottom) possible elongated shapes which can be due to a change in shape of the aggregates at high concentration. The bar 100 nm. Larger dark spots on the pictures are ice which has crystallized during the process.

TEM micrographs of a 1 wt % solution of NaPSS (molecular weight 70 000) reveal no aggregates in distinct contrast to the aggregates observed in the micrographs of the diblock copolymer solution. Precise sizes cannot be determined from the objects in the micrographs since underfocus is used to enhance image contrast (phase contrast). However the measured sizes of the dark spots on Figure 3a are consistent with the core diameter of 7 nm which has been determined by neutron scattering on diblock solutions.[5]

Both spherical and elongated or wormlike aggregates can be seen in the micrographs at a higher concentration of 1 wt %. The elongated objects appear to be on the order of 30 nm long and can be interpreted either as cylindrical micelles or as the two-dimensional projection of separate spherical objects. Since the concentration of 1 wt % is close to $c^*$, micelles should begin interacting strongly. This interaction may lead either to an actual change in the shape of the core or to the apparent formation of elongated objects due to their proximity. Both explanations are equally valid (and nonexclusive) about the modification of the hydrodynamic radius as deduced from NMR measurements.

Following the first explanation, a change in shape of the core could reflect a change in the overall shape of the micelle toward a more extended shape. The quantity $k_BT/6\pi\eta D$ would then provide a measurement of the more extended dimension (to a numerical factor[19]) which could explain the discrepancy between the measurements at 1 wt % and in the dilute regime. Recent theoretical calculations[20] indicate such possibilities of cylindrical shapes of micelles for charged diblock copolymers as a function of salt concentration but the influence of the polymer concentration has not been studied so far. Neutron scattering experiments selectively sensitive to the micellar cores should be able to confirm any appreciable change when concentration is increased through $c^*$. Such experiments are currently in progress.

The second explanation relies on the existence of interactions between micelles due to their proximity. It must be noted that $c^*$ is not very sensitive to the addition of electrolyte since the overall contraction of the micelles is weak (Figure 2). Then, even at the salinity where NMR experiments were performed, appreciable interactions may exist between micelles at 1 wt %, leading to a decrease of the self-diffusion coefficient $D_s$ and to an apparent increase in the hydrodynamic radius. These interactions are also clearly visible in total scattering intensity measurements (neutron or X-ray) where they lead to correlation peaks.[21]

## Conclusion

In conclusion we believe that the results of quasielastic light scattering, fluorescence recovery after photobleaching, pulsed field gradient spin−echo NMR spectroscopy and cryo-transmission electron microscopy unequivocally establish the existence in aqueous solution of polyelectrolyte micelles of the diblock copolymer surfactant studied herein. Moreover these techniques have proven to be quantitatively useful for characterizing such complicated systems as charged copolymers in aqueous solutions. In particular we showed here that a fluorescent probe can be efficiently linked to to a polymeric micelle, thus leading to quantitative information. The results are quantitatively consistent in the dilute regime although the measured quantities are of different origin.

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